IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Himanshu BRAHMBHATT *et al.*

Title: **PHARMACEUTICALLY COMPATIBLE METHOD FOR PURIFYING INTACT BACTERIAL MINICELLS**

Appl. No.: 10/602,021

Filing Date: 6/24/2003

Examiner: Leon B. Lankford, Jr.

Art Unit: 1651

Confirmation Number: 7643

AMENDMENT AND REPLY UNDER 37 C.F.R. § 1.116

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Sir:

This paper is a response to the non-final Office Action mailed May 15, 2007 concerning the captioned patent application.

Amendments to the Claims are reflected in the listing of claims that begins on page 2 of this document.

Remarks/Arguments begin on page 5 of this document.

AMENDMENTS TO THE CLAIMS

This listing replaces all prior versions and listings of claims in the application.

1-8. (Canceled)

9. (Currently Amended) A purification method for separating minicells from parent bacterial cells that comprises (a) subjecting a sample enriched for minicells to a condition selected from the group consisting of a stress-inducing osmotic condition, an anaerobic condition and a nutrient-limiting condition, which condition induces parent bacterial cells to adopt a filamentous form, and then (b) filtering said sample, wherein said filtering passes minicells but not filamentous parent bacterial cells, such that said method yields a purified composition of minicells.

10. (Canceled)

11. (Original) A method according to claim 9, wherein said sample is incubated in a hypertonic medium.

12. (Original) A method according to claim 9, wherein the filtering step is a dead-end filtration with a filter employing a pore size of about 0.45 μm.

13-26. (Canceled)

27. (Previously Presented) A method according to claim 9, wherein the filtering step comprises cross-flow filtration.

28. (Previously Presented) A method according to claim 9, wherein the filtering step comprises a serial filtration process that combines cross-flow filtration and dead-end filtration.

29. (Previously Presented) A method according to claim 28, wherein the filtering step employs at least one filter employing a pore size less than or equal to about 0.2 μm.

30. (Previously Presented) A method according to claim 28, wherein the filtering step employs at least one filter employing a pore size greater than or equal to about 0.45 μm.

31. (Previously Presented) A method according to claim 28, wherein said serial filtration process is preceded by differential centrifugation.

32. (Previously Presented) A method according to claim 9, wherein the filtering step employs at least one filter employing a pore size less than or equal to about 0.2 μm.

33. (Previously Presented) A method according to claim 9, wherein the filtering step employs at least one filter employing a pore size greater than or equal to about 0.45 μm.

34. (Previously Presented) A method according to claim 9, further comprising a step of subjecting the minicells to density gradient centrifugation in a biologically compatible medium.

35. (Previously Presented) A method according to claim 34, further comprising a step of subjecting the minicells to differential centrifugation.

36. (Previously Presented) A method according to claim 34, wherein said medium is isotonic and non-toxic.

37. (Previously Presented) A method according to claim 34, wherein said medium consists essentially of iodixanol and water.

38. (Previously Presented) A method according to claim 9, further comprising a step of treating said purified composition of minicells with an antibiotic.

39. (Previously Presented) A method according to claim 9, further comprising a step of removing free endotoxin from said purified composition of minicells.

40. (Previously Presented) A method according to claim 39, wherein said step of removing free endotoxin employs anti-Lipid A antibodies.

41. (New) A method according to claim 9, wherein the purified composition of minicells contains fewer than about 1 contaminating parent bacterial cell per 10^7, 10^8, 10^9, 10^{10} or 10^{11} minicells.

42. (New) A method according to claim 9, wherein the purified composition of minicells contains fewer than about 1 contaminating parent bacterial cell per 10^9 minicells.

43. (New) A method according to claim 9, wherein the purified composition of minicells contains fewer than about 1 contaminating parent bacterial cell per 10^{10} minicells.

44. (New) A method according to claim 9, wherein the purified composition of minicells contains fewer than about 1 contaminating parent bacterial cell per 10^{11} minicells.

REMARKS

Applicants respectfully request reconsideration of this application in view of the foregoing amendments and the following remarks.

I. STATUS OF THE CLAIMS

An amendment to claim 9 is proposed. Claims 41-44 have been added. Support for these new claims can be found at least in paragraph [0021] and original claims 20-24. Upon entry of the amendments, claims 9, 11-12 and 27-44 will be pending in the application.

II. REJECTIONS UNDER 35 U.S.C. § 112, ¶2

The examiner rejects claims 9, 11-12 and 27-40 for alleged indefiniteness. According to the rejection, the term "parent bacterial cells" is unclear. While applicants respectfully disagree, the amendment proposed by the examiner will not impact the scope of the pending claims. Accordingly, applicants have revised the claims in line with the examiner's suggestion, which, they submit, should obviate the rejection.

III. REJECTIONS UNDER 35 U.S.C. § 103

The examiner rejects claims 9-12 and 26-40 under 35 U.S.C. § 103 for allegedly being unpatentable over Khatchatourians et al. Applicants respectfully traverse the rejection.

Khatchatourians is cited for allegedly "teach[ing] the separation of minicells from normal, contaminating bacterial cells by inducing normal cells to filamentate followed by selective elimination of the filamentous bacteria." Office Action dated May 15, 2007, pg. 3.

Khatchatourians actually taught (a) using low levels of penicillin to inhibit cell division but not longitudinal growth of *E. coli* cells and then (b) selectively eliminating filamentous bacteria by sonic oscillation of whole cells, followed by centrifugation purification. Khatchatourians, Discussion, ¶¶1-2 (Materials and Methods, "Preparation of minicells"). In proposing his method, Khatchatourians assumed that "sonic treatment disrupts whole cells" and "does not affect minicells." *Id.* at 293. This assumption proved to be false, however. In the decades following Khatchatourians' 1973 publication, practitioners learned that sonication seriously damages minicells as well as bacterial cells. In fact,

sonication became the standard method of minicell disruption in the 1980's and 1990's. Evidencing this fact is Henning et al., *Proc. Nat. Acad. Sci. USA* 76: 4360-64 (1979) (copy appended).

Thus, Khatchatourians' notion of preparing preparations of minicells is fundamentally flawed, as is the examiner's rationale for rejection that is based on this reference. The skilled artisan, *circa* 2003, would have been well-aware of Khatchatourians' error and, hence, would have dismissed the Khatchatourians methodology as illustrating how *not* to purify bacterial minicells.

If anything, therefore, Khatchatourians teaches away from the presently claimed methodology and, certainly, cannot render applicants' claims obvious within the meaning of Section 103. Furthermore, the examiner has acknowledged that Khatchatourians fails to teach the use of filtration to remove filamentous parent bacterial cells. In the absence of any suggestion on point, the examiner asserts that it would have been obvious for the skilled artisan simply to replace Khatchatourians' centrifugation purification step with "available filters." Again, this assertion is fundamentally flawed as a matter of fact.

The use of filtration requires knowledge of the size and size uniformity of the particles to be purified. Neither of these prerequisites was known prior to applicants invention. Before applicants disclosure, in other words, the skilled artisan would have had no reason to exchange Khatchatourians' centrifugation purification step with "available filters" and, in any event, would have had no principled basis for expecting success from such an exchange.

Applicants were the first to determine that minicells have a diameter of approximately 400 nm. Moreover, despite the well-known heterogeneity of bacterial cell diameters, applicants discovered that minicells from a diverse range of bacteria, *e.g.* Gram negative to Gram positive bacteria, possess a uniform diameter. With these two discoveries in hand, applicants showed that the claimed methods could be employed to separate minicells from filamentous parent bacterial cells to yield a composition of minicells at previously unattainable levels of purity.

Applicants believe, therefore, that the examiner has failed to establish a *prima facie* case of obviousness and request that the rejection be withdrawn.

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Applicants submit that this application is allowable condition and request an early indication to that effect. The examiner is invited to contact the undersigned directly, should he feel that any issue requires further consideration.

The Commissioner is hereby authorized to charge any additional fees, which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, and to credit any overpayment to Deposit Account No. 19-0741. Should no proper payment accompany this response, then the Commissioner is authorized to charge the unpaid amount to the same deposit account. If any extension is needed for timely acceptance of submitted papers, then applicants hereby petition for such extensions under 37 CFR §1.136 and authorize payment of the relevant fee(s) from the deposit account.

Respectfully submitted,

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